Genetic variation in *N*-acetyltransferase 1 (*NAT1*) and 2 (*NAT2*) and risk of non-Hodgkin lymphoma

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Animal studies suggest that lymphomagenesis can be induced by exposure to carcinogenic aromatic and heterocyclic amines found in diet, cigarette smoke and the environment, but human epidemiologic investigations of these exogenous exposures have yielded conflicting results. As part of our evaluation of the role of aromatic and heterocyclic amines, which are metabolized by *N*-acetyltransferase (NAT) enzymes, in the etiology of non-Hodgkin lymphoma (NHL), we examined NHL risk in relation to genetic variation in *NAT1* and *NAT2* and exposure to cigarette smoke and dietary heterocyclic amines and mutagens. We genotyped 10 common single nucleotide polymorphisms (SNPs) in *NAT1* and *NAT2* among 1136 cases and 922 controls from a population-based case–control study in four geographical areas of the USA. Relative risk of NHL for *NAT1* and *NAT2* genotypes, *NAT2* acetylation phenotype, and exposure to cigarette smoke and dietary heterocyclic amines and mutagens was estimated using odds ratios (ORs) and 95% confidence intervals (CIs) derived from unconditional logistic regression models. We observed increased risk of NHL among individuals with the *NAT1*10/*10* genotype compared with individuals with other *NAT1* genotypes (OR=1.60, 95% CI=1.04–2.46, *P*=0.03). We also observed increased NHL risk in a dose-dependent model among *NAT2* intermediate- and rapid-acetylators compared with slow-acetylators, although only the trend was statistically significant (intermediate: OR=1.18, 95% CI=0.97–1.44, *P*=0.1; rapid: OR=1.43, 95% CI=0.97–2.14, *P*=0.07; *P* for linear trend =0.03). Compared with non-smokers, NHL risk estimates for current cigarette smoking were increased only among *NAT2* intermediate/rapid-acetylators (OR=2.44, 95% CI=1.15–5.20, *P*=0.02). Our data provide evidence that *NAT1* and *NAT2* genotypes are associated with NHL risk and support a contributory role for carcinogenic aromatic and/or heterocyclic amines in the multi-factorial etiology of NHL. *Pharmacogenetics and Genomics* 16:537–545 © 2006 Lippincott Williams & Wilkins.

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Introduction

Non-Hodgkin lymphoma (NHL) comprises a group of closely related yet heterogeneous diseases characterized by the malignant transformation of lymphoid cells [1]. Well-established risk factors for NHL include certain infections, as well as treatments and diseases that cause severe immunosuppression [2]; however, the etiology of NHL remains largely unknown. Although animal studies have demonstrated that lymphomagenesis can be induced by exposure to various carcinogenic aromatic and heterocyclic amines found in diet from intake of well-done meats cooked at high-temperatures, cigarette smoke, and the environment [3–5], human epidemiologic investigations of NHL in relation to these exogenous exposures have yielded conflicting results [6–11]. Because aromatic and heterocyclic amines exhibit carcinogenic activity upon metabolism, it is plausible that genetic variation in aromatic and heterocyclic amine metabolism may modulate NHL risk.

N-acetyltransferase (NAT) enzymes are responsible for metabolizing aromatic and heterocyclic amines via

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N-acetylation, which generally leads to detoxification for monocyclic aromatic amines, or via *O*-acetylation, which leads to activation and the formation of DNA adducts linked to the induction of cancer [12,13]. Two distinct NAT enzymes, NAT1 and NAT2, have been identified, both of which are encoded by highly polymorphic genes located on chromosome 8 (8p23.1-p21.3 and 8p22, respectively) [14]. NAT1 and NAT2 appear to demonstrate some substrate specificity and varying levels of expression in different tissues [12,13]. Well-established sequence variation that corresponds to variation in acetylation capacity, and thus carcinogen metabolism, has been identified for NAT2 [13,15], but investigation of genetic variation in *NAT1* and *NAT2* in relation to NHL risk has been limited and inconsistent to date [16–19].

Differential susceptibility to NHL according to variation in acetylation capacity would provide important evidence supporting the role of carcinogenic aromatic and/or heterocyclic amines in the etiology of NHL. We therefore investigated NHL risk in relation to genetic variation in *NAT1* and *NAT2* and exposure to cigarette smoke and dietary heterocyclic amines and mutagens in a recently completed, population-based case–control study in the USA.

Methods

Study population

The study population has been described previously in detail [20]. Briefly, cases included 1321 patients with newly diagnosed NHL identified in four Surveillance, Epidemiology, and End Results (SEER) registries (state of Iowa and the metropolitan areas of Detroit, Michigan; Los Angeles, California; and Seattle, Washington) aged 20–74 years between July 1998 and June 2000 without evidence of HIV infection. Population controls ($n = 1057$) were identified by random digit dialing (under age 65 years) and from Medicare eligibility files (65 years and older). Participation and response rates were 76% and 59%, respectively, in cases, and 52% and 44%, respectively, in controls. The study was conducted according to a protocol approved by Institutional Review Boards at the National Cancer Institute and each of the four SEER centers. Written informed consent was obtained from each participant prior to interview. All study participants were asked to provide a venous blood or mouthwash buccal cell sample that was shipped to the biological repository for processing and storage. We obtained blood samples from 773 cases and 668 controls, and buccal cell samples from 399 cases and 314 controls; 149 (11%) cases and 75 (7%) controls provided neither. Demographic characteristics (age, education, sex) for individuals who provided blood compared with buccal cells and compared with those who provided neither blood, nor buccal cell samples were equivalent within each study site [21].

Histopathology

Each SEER registry provided NHL pathology and subtype information derived from abstracted reports by the local diagnosing pathologist. All cases were histologically confirmed and coded according to the International Classification of Diseases for Oncology, 2nd Edition (ICD-O-2) [22]. We evaluated the following histological outcomes: (i) NHL overall (ICD-O-2: 9590-01, 9595, 9670-73, 9675-76, 9680-88, 9690–91, 9695–98, 9700, 9702–03, 9705–11, 9713-15, 9823, 9827); (ii) B-cell lymphomas (ICD-O-2: 9670-71, 9673, 9676, 9680-88, 9690-91, 9695-98, 9710-11, 9715, 9823); (iii) T-cell lymphomas (ICD-O-2: 9700, 9702-03, 9705-09, 9713-14, 9827), and four B-cell lymphoma subtypes: (iv) diffuse large B-cell (DLBCL) (ICD-O-2: 9680-84, 9688); (v) follicular (ICD-O-2: 9676, 9690-91, 9695-98); (vi) marginal zone (ICD-O-2: 9710-11, 9715); and (vii) small lymphocytic lymphoma (SLL) (ICD-O-2: 9670-71, 9823).

Laboratory methods

DNA was extracted from blood clots or buffy coats (from 10 ml blood) at BBI Biotech Repository (Gaithersburg, Maryland, USA) using Puregene Autopure DNA extraction kits (Gentra Systems, Minneapolis, Minnesota, USA). DNA was extracted from buccal cell samples by phenol chloroform extraction methods [23].

We selected for genotyping four single nucleotide polymorphisms (SNPs) in *NAT1* and six SNPs in *NAT2* (Table 1). Data from the *NAT1* and *NAT2* SNPs genotyped in this study were used to assign the most likely *NAT1* and *NAT2* alleles previously identified in human populations using consensus nomenclature (http://www.louisville.edu/medschool/pharmacology/NAT.html) [24]. All 10 SNPs were genotyped in blood-based DNA samples first. Three SNPs (rs1041983, rs1799929 and rs1208) that were not informative for *NAT2* phenotype assignment in our study population were not genotyped in buccal cell samples, which generally had less DNA than blood-based samples (Table 1). Genotyping was

Table 1 ***N*-acetyltransferase 1 (*NAT1*) and 2 (*NAT2*) polymorphisms selected for genotyping**

SNP rs #	Nucleotide change[a]	Amino acid change
NAT1		
rs4987076	Ex1 + 591G > A	V149I
rs13249533	Ex1 – 616G > A	R187Q
rs1057126	Ex1 – 88T > A	–
rs15561	Ex1 – 81C > A	–
NAT2		
rs1041983[b]	Ex2 + 288C > T	Y94Y
rs1801280	Ex2 + 347T > C	I114T
rs1799929[b]	Ex2 + 487C > T	L161L
rs1799930	Ex2 – 580G > A	R197Q
rs1208[b]	Ex2 – 367A > G	K268R
rs1799931	Ex2 – 313G > A	G286E

[a]http://snp500cancer.nci.nih.gov [25].
[b]Genotyped in blood-based DNA samples only.

conducted at the National Cancer Institute Core Genotyping Facility (Advanced Technology Center, Gaithersburg, Maryland, USA) using validated assays on the Taqman (Applied Biosystems, Foster City, California, USA) or MGB Eclipse (Epoch Biosciences, Bothell, Washington, USA) platforms. Sequence data and assay conditions available at: http://snp500cancer.nci.nih.gov [25].

For the purposes of quality control (QC), 40 replicate samples from each of two blood donors and duplicate samples from 100 study participants processed in an identical fashion were interspersed for all genotyping assays and blinded from the laboratory. For each plate of 368 samples, genotype-specific QC samples were also included and comprised four homozygote wild-type, four heterozygote, four homozygote variant and four DNA negative controls. Finally, each individual was identified using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, California, USA). Of the 2154 study participants who donated a biological sample, 26 (3%) controls and 22 (2%) cases whose chromosomal sex based on the Identifiler was discordant from that reported during the interview were excluded from this analysis. Participants with insufficient DNA for this analysis or whose DNA was not successfully extracted and quantified [14 (1%) cases and 34 (3%) controls] were also excluded, yielding a final population of 1136 cases and 922 controls for genotyping and analyses of *NAT1* and *NAT2* SNPs in relation to NHL. Because of incomplete genotyping for some SNPs due to insufficient DNA, the most likely *NAT* alleles could not be determined for 220 (19%) cases and 176 (19%) controls for *NAT1*, and 151 (13%) cases and 114 (12%) controls for *NAT2*, yielding a final population of 916 cases and 746 controls for analyses of *NAT1* genotypes, and 985 cases and 808 controls for analyses of *NAT2* genotypes and phenotypes. Demographic characteristics (age, education, sex) for cases and controls did not differ significantly for individuals with undetermined *NAT* alleles compared with those with known *NAT* alleles.

For nine of 10 assays (excepting one *NAT1* SNP, rs15561), genotyping was completed successfully in 95–100% of the DNA samples and agreement for QC replicates and duplicates was $>97\%$. Completion rate for rs15561 was 83% in blood-based DNA; however, quality control data were rechecked and the assay was deemed to be acceptable. The genotype frequencies among white, non-Hispanic controls were in Hardy–Weinberg equilibrium for all 10 SNPs (Pearson $\chi^2 > 0.05$).

Exposure assessment

The study population was divided into two groups, each receiving a different version of a computer-assisted personal interview and a different self-administered questionnaire. The present analysis of history of cigarette smoking and exposure to dietary heterocyclic amines and mutagens is thus based on the 484 cases and 419 controls who returned the self-administered dietary questionnaire.

Details have been published previously for the exposure assessment of history of cigarette smoking [9] and dietary exposure to heterocyclic amines [8]. Briefly, participants were classified as non-smokers and used as the referent group for all analyses if they had never smoked regularly for at least 6 months. Former smokers were defined as those who stopped smoking at least 1 year prior to diagnosis (for cases) or interview (for controls). All smokers were asked to provide additional information about the age they began smoking, and the duration and intensity (cigarettes/day) of smoking. Dietary exposure to three heterocyclic amines, including 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), was derived from information on the frequency of consumption, portion size, cooking method, and 'doneness' level for hamburger, steak, pork chops, bacon, and sausage linked to a database on heterocyclic amines (http://charred.cancer.gov). This database was also used to create a continuous measure of the total mutagenic potential from meat consumption. All four variables were classified into quintiles according to the distribution among controls, and the first quintile was used as the referent group for all analyses.

Statistical analysis

Variation in demographic characteristics for cases and controls was assessed using the Pearson chi-square statistic. To estimate the relative risk of NHL associated with *NAT1* and *NAT2* genotypes, history of cigarette smoking, and exposure to dietary heterocyclic amines and mutagens, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) derived from dichotomous multivariate unconditional logistic regression models. We also calculated a *P*-value for the linear trend based on a three-level ordinal variable for each genotype (0 = homozygote common, 1 = heterozygote, 2 = homozygote variant). Heterogeneity among NHL subtypes was assessed in polytomous multivariate unconditional logistic regression models using the Wald chi-square statistic.

For analyses of *NAT1*, *NAT1*10* was designated as the at-risk allele based on previous research [15]. We thus calculated ORs, 95% CIs, and a *P*-value for the linear trend comparing individuals with one or two copies of *NAT1*10* with individuals with no copies of *NAT1*10*. For analyses of *NAT2*, we designated *NAT2*4/*4* as the referent genotype, as done previously, because it corresponds to an absence of the nucleotide substitutions

that define the other *NAT2* genotypes [15]. The *NAT2* genotype–phenotype relationship is well-established. NAT2 acetylation phenotype was assigned at the University of Louisville (by DWH) based on data obtained *in vivo* and *in vitro* on allelic variation in catalytic activity [13]. For analysis of NAT2 phenotypes, individuals homozygous for *NAT2* rapid- and slow-acetylator alleles were designated as rapid- and slow-acetylators, respectively; individuals possessing one rapid- and one slow-acetylator allele (heterozygotes) were designated as intermediate-acetylators [26].

All logistic regression models were adjusted for sex, age (<45, 45–64, 65+ years), race (white non-Hispanic, black, other), and study center because these were study design variables. Additional adjustment for education (high school or less, some college, college graduate) and cigarette smoking (current/former/never, cigarettes per day or duration) did not materially alter ($>10\%$) the parameter estimates for *NAT1* or *NAT2*; these variables were therefore excluded from the final model. Models for exposure to dietary heterocyclic amines and mutagens were also adjusted for physical activity (none, 30–270, 271–675, 676–1080, >1080 metabolic equivalent tasks per week), total caloric intake (kcal/day), alcohol consumption (<1,1–15, >15–30, >30 g/day), and animal protein (g/day), based on previous analyses of these data [8]. Modification of the effect of *NAT1* and *NAT2* on risk of NHL by sex, race, cigarette smoking, and dietary exposures was evaluated under the multiplicative model using a Wald chi-square statistic by including an interaction term in the logistic regression models. The SAS System, version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA), was used to conduct all statistical analyses. *P*-values are presented with one significant digit. Statistical tests were two-sided with an α-level of 0.05.

Results

Table 2 presents selected characteristics for the study population. Cases were younger and more likely to be white, non-Hispanic than controls. Cases and controls did not differ significantly with respect to sex, history of cigarette smoking, or exposure to dietary heterocyclic amines and mutagens. DLBCL and follicular lymphoma accounted for slightly more than half of the histological subtypes for cases.

The prevalence of the *NAT1*10/*10* genotype among controls was 5.5%. We observed increased risk of NHL among individuals with two copies of *NAT1*10* compared with individuals with no copies of *NAT1*10* (**10/any*: OR = 1.07, 95% CI = 0.87–1.33, $P = 0.5$; **10/*10*: OR = 1.60, 95% CI = 1.04–2.46, $P = 0.03$; P for linear trend = 0.07) (Table 3). Risk estimates were generally increased for all the NHL subtypes evaluated, although higher risk estimates were observed for follicular lymphoma than for other subtypes (**10/*10*: OR = 2.06, 95% CI = 1.12–3.79, $P = 0.02$). The results from our analyses of other *NAT1* genotypes and SNPs supported our designation of *NAT1*10* as the at-risk allele. Analyses of other *NAT1* genotypes in comparison with the *NAT1*4/*4* genotype demonstrated increased risk of NHL for *NAT1*10/*10* only (OR = 1.61, 95% CI = 1.04–2.48, $P = 0.03$) (Table 4).

In the analyses by *NAT2* phenotype, an increased risk of NHL was observed in a dose-dependent model among intermediate- and rapid-acetylators in comparison with

Table 2 Selected characteristics of cases ($n=1136$) and controls ($n=922$) with *NAT1* and *NAT2* single nucleotide polymorphism genotype data in the NCI-SEER multicenter case–control study of non-Hodgkin lymphoma

	Cases		Controls		
	n	(%)	*n*	(%)	*P*[a]
Sex					
Male	622	(54.8%)	487	(52.8%)	0.4
Female	514	(45.2%)	435	(47.2%)	
Age (years)					
<45	200	(17.6%)	144	(15.6%)	0.0006
45–64	561	(49.4%)	398	(43.2%)	
65+	375	(33.0%)	380	(41.2%)	
Race					
White, non-Hispanic	980	(86.3%)	743	(80.6%)	<0.0001
Black	78	(6.9%)	124	(13.4%)	
Other	78	(6.9%)	55	(6.0%)	
Study center					
Detroit	232	(20.4%)	160	(17.4%)	0.1
Iowa	333	(29.3%)	257	(27.9%)	
Los Angeles	282	(24.8%)	238	(25.8%)	
Seattle	289	(25.4%)	267	(29.0%)	
History of cigarette smoking					
Non-smoker	208	(18.3%)	163	(17.7%)	0.1[b]
Current smoker	80	(7.0%)	55	(6.0%)	
Former smoker	148	(13.0%)	147	(15.9%)	
Missing	700	(61.6%)	557	(60.4%)	
Mutagenic potential (revertant colonies/day)					
Q1: 0.0–1700.0	79	(7.0%)	64	(6.9%)	0.2[b]
Q2: 1700.1–3416.1	68	(6.0%)	74	(8.0%)	
Q3: 3416.2–6470.0	99	(8.7%)	68	(7.4%)	
Q4: 6470.1–11 338.0	103	(9.1%)	73	(7.9%)	
Q5:>11 338.0	76	(6.7%)	69	(7.5%)	
Missing	711	(62.6%)	574	(62.3%)	
DNA source					
Blood	751	(66.1%)	639	(69.3%)	0.1
Buccal cells	385	(33.9%)	283	(30.7%)	
Case histopathology					
DLBCL	358	(31.5%)			
Follicular lymphoma	269	(23.7%)			
SLL	146	(12.9%)			
Marginal zone	91	(8.0%)			
Mantle cell	41	(3.6%)			
Burkitt lymphoma	18	(1.6%)			
All T-cell	70	(6.2%)			
NOS	143	(12.6%)			

[a]*P*-value for Pearson chi-square statistic.
[b]*P*-values computed excluding those with missing data for history of cigarette smoking and diet. DLBCL, diffuse large B-cell; SLL, small lymphocytic lymphoma; NOS, not otherwise specified.

Table 3 Association between *NAT1*10* and risk of non-Hodgkin lymphoma (NHL) and NHL subtypes

	*NAT1*any(except *10)/*any (except *10)*			*NAT1*10/*any(except *10)*				*NAT1*10/*10*			*P* for linear trend	*NAT1*10/any(except *10) | NAT1*10/*10*			
	n (%)	OR	(95% CI)[a]	*n* (%)	OR	(95% CI)[a]	*P*	*n* (%)	OR	(95% CI)[a]	*P*		*n* (%)	OR	(95% CI)[a]	*P*
Controls	454 (60.9)			251 (33.6)				41 (5.5)					292 (39.1)			
All NHL	547 (59.7)	1.00	(reference)	303 (33.1)	1.07	(0.87–1.33)	0.5	66 (7.2)	1.60	(1.04–2.46)	0.03	0.07	369 (40.3)	1.14	(0.92–1.40)	0.2
By NHL subtype																
DLBCL	167 (57.4)	1.00	(reference)	106 (36.4)	1.25	(0.93–1.68)	0.1	18 (6.2)	1.49	(0.81–2.74)	0.2	0.08	124 (42.6)	1.28	(0.96–1.70)	0.09
Follicular lymphoma	138 (63.9)	1.00	(reference)	59 (27.3)	0.87	(0.62–1.24)	0.5	19 (8.8)	2.06	(1.12–3.79)	0.02	0.3	78 (36.1)	1.01	(0.73–1.40)	0.9
SLL	71 (59.7)	1.00	(reference)	43 (36.1)	1.21	(0.79–1.84)	0.4	5 (4.2)	0.96	(0.36–2.57)	0.9	0.6	48 (40.3)	1.18	(0.79–1.77)	0.4
Marginal zone	41 (57.7)	1.00	(reference)	25 (35.2)	1.08	(0.64–1.84)	0.8	5 (7.0)	1.51	(0.55–4.17)	0.4	0.5	30 (42.3)	1.13	(0.68–1.88)	0.6
All T-cell	33 (61.1)	1.00	(reference)	16 (29.6)	0.93	(0.49–1.76)	0.8	5 (9.3)	1.84	(0.64–5.27)	0.3	0.6	21 (38.9)	1.04	(0.58–1.89)	0.9

[a]Odds ratio (OR) [95% confidence interval (CI)] adjusted for age, sex, race, and study center. DLBCL, diffuse large B-cell; SLL, small lymphocytic lymphoma.

slow-acetylators, although only the trend was statistically significant (intermediate: OR = 1.18, 95% CI = 0.97–1.44, $P = 0.1$; rapid: OR = 1.44, 95% CI = 0.97–2.14, $P = 0.07$; P for linear trend = 0.03) (Table 5). Risk estimates were generally increased for all the NHL subtypes evaluated, although the increased risk was most pronounced for follicular lymphoma (intermediate/rapid-acetylators: OR = 1.50, 95% CI = 1.11–2.02, $P = 0.008$) and for marginal zone lymphoma (intermediate/rapid-acetylators: OR = 1.56, 95% CI = 0.98–2.49, $P = 0.06$). Further analysis of *NAT2* genotypes supported our finding of increased risk among rapid-acetylators; the NHL risk estimates for the *NAT2* genotypes corresponding to the intermediate- and slow-acetylator phenotypes in comparison with those corresponding to the rapid-acetylator phenotype were consistently less than 1.0 (Table 6).

In our study population, *NAT1*10* was over-represented among *NAT2* rapid-acetylators (frequency of *NAT1*10/*10* was 3%, 7%, and 20% in *NAT2* slow-, intermediate- and rapid-acetylators, respectively; Pearson $\chi^2 < 0.0001$). However, inclusion of both *NAT1* and *NAT2* in the same model did not materially alter (> 10%) the NHL risk estimates for *NAT*10/*10* or *NAT2* rapid-acetylators, nor was there interaction between *NAT1* and *NAT2* (data not shown).

Although analyses stratified by sex revealed that risk estimates for *NAT1* genotypes and *NAT2* phenotypes were slightly higher for men than for women, these differences were not statistically significant under the multiplicative model (data not shown). *NAT1* genotype and *NAT2* phenotype frequencies differed between non-Hispanic whites and blacks; however, the NHL risk estimates were not statistically significantly different under the multiplicative model (Online Supplementary Table).

The risk of NHL associated with history of cigarette smoking differed significantly by *NAT2* phenotype (interaction $P = 0.05$), but not by *NAT1* genotype (interaction $P = 0.9$) under the multiplicative model (Table 7). Among *NAT2* intermediate-/rapid-acetylators, risk estimates for NHL were 2.4-fold for current smokers compared with non-smokers (OR = 2.44, 95% CI = 1.15–5.20, $P = 0.02$), whereas the risk of NHL was not related to current cigarette smoking among *NAT2* slow-acetylators (OR = 0.87, 95% CI = 0.50–1.53, $P = 0.6$). The risk of NHL associated with exposure to dietary heterocyclic amines and mutagens did not differ significantly by *NAT1* genotype or by *NAT2* phenotype under the multiplicative model (data not shown).

Discusssion

In this large, population-based case–control study, we report an increased risk of NHL among individuals with

Table 4 Association between *NAT1* genotypes and risk of non-Hodgkin lymphoma

NAT1 genotype	Controls *n* (%)	Cases *n* (%)	OR	(95% CI)[a]	*P*
*NAT1*4/*4*	386 (51.3)	463 (50.3)	1.00	(reference)	
*NAT1*10/*10*	42 (5.6)	68 (7.4)	1.61	(1.04–2.48)	0.03
*NAT1*4/*10*	206 (27.4)	254 (27.6)	1.11	(0.87–1.40)	0.4
*NAT1*4/*11A* or **3/*11B*	20 (2.7)	27 (2.9)	1.04	(0.57–1.90)	0.9
*NAT1*4/*3*	31 (4.1)	26 (2.8)	0.70	(0.40–1.21)	0.2
Other[b]	67 (8.9)	83 (9.0)	1.10	(0.77–1.57)	0.6

[a]Odds ratio (OR) [95% confidence interval (CI)] adjusted for age, sex, race, and study center.
[b]Other NAT1 genotypes (<2% prevalence among controls): *NAT1*10/*11A*, *NAT1*10/*14A*, *NAT1*11A/*14A*, *NAT1*14A/*14A*, *NAT1*3/*10*, *NAT1*3/*11A*, *NAT1*3/*14A*, *NAT1*4/*14A*, *NAT1*10/*14B*.

Table 5 Association between *NAT2* phenotypes and risk of non-Hodgkin lymphoma (NHL) and NHL subtypes

	Slow[a]		Intermediate[a]			Rapid[a]				Intermediate/Rapid		
	n (%)	OR (95% CI)[b]	*n* (%)	OR (95% CI)[b]	*P*	*n* (%)	OR (95% CI)[b]	*P*	*P* for linear trend	*n* (%)	OR (95% CI)[b]	*P*
Controls	452 (55.9)		306 (37.9)			50 (6.2)				356 (44.1)		
All NHL	523 (53.1)	1.00 (reference)	390 (39.6)	1.18 (0.97–1.44)	0.1	72 (7.3)	1.44 (0.97–2.14)	0.07	0.03	462 (46.9)	1.21 (1.00–1.47)	0.05
By NHL subtype												
DLBCL	173 (55.4)	1.00 (reference)	114 (36.5)	1.05 (0.79–1.39)	0.7	25 (8.0)	1.52 (0.89–2.58)	0.1	0.2	139 (44.6)	1.11 (0.85–1.45)	0.5
Follicular lymphoma	113 (48.5)	1.00 (reference)	104 (44.6)	1.50 (1.10–2.04)	0.01	16 (6.9)	1.52 (0.82–2.82)	0.2	0.01	120 (51.5)	1.50 (1.11–2.02)	0.008
SLL	73 (56.6)	1.00 (reference)	46 (35.7)	1.02 (0.68–1.52)	0.9	10 (7.8)	1.58 (0.75–3.31)	0.2	0.4	56 (43.4)	1.08 (0.74–1.59)	0.7
Marginal zone	37 (45.1)	1.00 (reference)	44 (53.7)	1.76 (1.10–2.82)	0.02	1 (1.2)	0.24 (0.03–1.83)	0.2	0.4	45 (54.9)	1.56 (0.98–2.49)	0.06
All T-cell	36 (63.2)	1.00 (reference)	18 (31.6)	0.78 (0.43–1.42)	0.4	3 (5.3)	0.86 (0.25–2.99)	0.8	0.5	21 (36.8)	0.79 (0.45–1.41)	0.4

[a]*NAT2* phenotypes: Slow (*NAT2*5B/*6A, NAT2*5/*5, NAT2*5/*6, NAT2*5B/*5B, NAT2*5B/*7B, NAT2*6/*6, NAT2*6A/*6A, NAT2*5/*7, NAT2*5A/*5B, NAT2*5A/*5C, NAT2*5A/*6A, NAT2*5A/*7B, NAT2*5B/*5C, NAT2*5C/*6A, NAT2*5C/*7B, NAT2*6/*7, NAT2*6A/*7B, NAT2*7/*7, NAT2*7B/*7B*); Intermediate (*NAT2*4/*5, NAT2*4/*5B, NAT2*4/*6, NAT2*4/*6A, NAT2*4/*5A, NAT2*4/*5C, NAT2*4/*7, NAT2*4/*7B, NAT2*5B/*12A, NAT2*5B/*13, NAT2*5C/*12A, NAT2*6A/*12A, NAT2*6A/*13, NAT2*7B/*12A, NAT2*7B/*13); Rapid (NAT2*4/*4, NAT2*12A/*13, NAT2*4/*12A, NAT2*4/*13*).
[b]Odds ratio (OR) [95% confidence interval (CI)] adjusted for age, sex, race, and study center. DLBCL, diffuse large B-cell; SLL, small lymphocytic lymphoma.

Table 6 Association between *NAT2* genotypes and risk of non-Hodgkin lymphoma

	Controls *n* (%)	Cases *n* (%)	OR	(95% CI)[a]	*P*	Acetylator phenotype
*NAT2*5B/*6A*	129 (16.0)	137 (13.9)	0.64	(0.40–1.01)	0.06	Slow
*NAT2*5/*5*	27 (3.3)	55 (5.6)	1.16	(0.63–2.13)	0.6	Slow
*NAT2*5/*6*	59 (7.3)	77 (7.8)	0.77	(0.45–1.30)	0.3	Slow
*NAT2*5B/*5B*	94 (11.6)	94 (9.6)	0.59	(0.36–0.97)	0.04	Slow
*NAT2*5B/*7B*	22 (2.7)	22 (2.2)	0.59	(0.29–1.20)	0.1	Slow
*NAT2*6/*6*	19 (2.4)	26 (2.6)	0.88	(0.43–1.81)	0.7	Slow
*NAT2*6A/*6A*	46 (5.7)	52 (5.3)	0.66	(0.38–1.17)	0.2	Slow
Other *NAT2* slow-acetylator genotypes[b]	56 (6.9)	59 (6.0)	0.67	(0.39–1.15)	0.1	Slow
*NAT2*4/*5*	45 (5.6)	57 (5.8)	0.88	(0.50–1.55)	0.7	Intermediate
*NAT2*4/*5B*	88 (10.9)	151 (15.3)	1.04	(0.65–1.68)	0.9	Intermediate
*NAT2*4/*6*	42 (5.2)	33 (3.4)	0.49	(0.27–0.90)	0.02	Intermediate
*NAT2*4/*6A*	92 (11.4)	92 (9.3)	0.61	(0.37–0.99)	0.04	Intermediate
Other *NAT2* intermediate-acetylator genotypes[b]	39 (4.8)	57 (5.8)	1.13	(0.64–2.01)	0.7	Intermediate
*NAT2*4/*4*	45 (5.6)	66 (6.7)	1.00	(reference)		Rapid
Other *NAT2* rapid-acetylator genotypes[b]	5 (0.6)	6 (0.6)	0.97	(0.27–3.49)	1.0	Rapid

[a]Odds ratio (OR) [95% confidence interval (CI)] adjusted for age, sex, race, and study center.
[b]Other *NAT2* slow-acetylator genotypes (<2% prevalence among controls): *NAT2*5/*7, NAT2*5A/*5B, NAT2*5A/*5C, NAT2*5A/*6A, NAT2*5A/*7B, NAT2*5B/*5C, NAT2*5C/*6A, NAT2*5C/*7B, NAT2*6/*7, NAT2*6A/*7B, NAT2*7/*7, NAT2*7B/*7B.* Other NAT2 intermediate-acetylator genotypes (<2% prevalence among controls*): NAT2*4/*5A, NAT2*4/*5C, NAT2*4/*7, NAT2*4/*7B, NAT2*5B/*12A, NAT2*5B/*13, NAT2*5C/*12A, NAT2*6A/*12A, NAT2*6A/*13, NAT2*7B/*12A, NAT2*7B/*13*. Other *NAT2* rapid-acetylator genotypes (<2% prevalence among controls): *NAT2*12A/*13, NAT2*4/*12A, NAT2*4/*13*.

the *NAT1*10/*10* genotype, present in 5.5% of controls, compared with other *NAT1* genotypes. Risk of NHL was also higher among individuals with *NAT2* rapid-acetylator genotypes, present in 6.2% of controls, compared with those with slow-acetylator genotypes. The risk of NHL associated with current cigarette smoking was observed

Table 7 Association between history of cigarette smoking and risk of non-Hodgkin lymphoma by *NAT1*10* and *NAT2* phenotype

	*NAT1*any(except *10)/*any(except *10)*					*NAT1*10/any(except *10)|NAT1*10/*10*				
	Controls *n* (%)	Cases *n* (%)	OR	(95% CI)[a]	*P*	Controls *n* (%)	Cases *n* (%)	OR	(95% CI)[a]	*P*
Non-smokers	85 (18.7)	102 (18.6)	1.00	(reference)		43 (14.7)	62 (16.8)	1.00	(reference)	
Current smokers	30 (6.6)	42 (7.7)	1.12	(0.64–1.95)	0.7	14 (4.8)	28 (7.6)	1.32	(0.62–2.84)	0.5
Former smokers	78 (17.2)	74 (13.5)	0.86	(0.55–1.33)	0.5	36 (12.3)	46 (12.5)	0.85	(0.47–1.56)	0.6
Missing	261 (57.5)	329 (60.1)	1.11	(0.79–1.57)	0.6	199 (68.2)	233 (63.1)	0.95	(0.60–1.49)	0.8
	[*NAT2 Slow*]					*Intermediate/Rapid*				
	Controls *n* (%)	Cases *n* (%)	OR	(95% CI)[a]	*P*	Controls n (%)	Cases n (%)	OR	(95% CI)[a]	*P*
Non-smokers	82 (18.1)	93 (17.8)	1.00	(reference)		60 (16.9)	88 (19.0)	1.00	(reference)	
Current smokers	34 (7.5)	36 (6.9)	0.87	(0.50–1.53)	0.6	11 (3.1)	38 (8.2)	2.44	(1.15–5.20)	0.02
Former smokers	70 (15.5)	75 (14.3)	0.99	(0.63–1.55)	0.9	56 (15.7)	57 (12.3)	0.70	(0.42–1.15)	0.2
Missing	266 (15.5)	319 (61.0)	1.06	(0.74–1.50)	0.8	229 (64.3)	279 (60.4)	1.03	(0.70–1.52)	0.9

[a]Odds ratio (OR) [95% confidence interval (CI)] adjusted for age, sex, race, and study center.

only among *NAT2* intermediate- and rapid-acetylators. The observed relationship between variation in acetylation capacity and differential susceptibility to NHL indirectly supports the role of carcinogenic aromatic and/or heterocyclic amines in the etiology of NHL.

Sources of exposure to aromatic or heterocyclic amines include cigarette smoke, dietary intake of well-done meats cooked at high-temperatures, hair dyes, and occupations within industries using aniline. Aromatic and heterocyclic amines such as these are bioactivated in a two-step process that includes oxidation by cytochrome P450 enzymes and subsequent *O*-acetylation by NAT1 and/or NAT2 [15,27]. The resulting acetoxy-ester intermediates are capable of forming DNA adducts [12,13,15,28] and have been linked to the induction of various cancers in rodents, including lymphomas [3,5,29]. However, human epidemiologic investigations of NHL in relation to aromatic or heterocyclic amine exposures have yielded conflicting results [6–11]. We present the first evidence that the potential relationship between NHL risk and these exogenous exposures may be modified by common genetic variation in acetylation capacity. We observed an increased risk of NHL associated with current cigarette smoking among *NAT2* intermediate-/rapid-acetylators, which supports a role for diarylamines or heterocyclic amines that are present at higher concentrations in cigarette smoke than in well-done meats in the etiology of NHL. Future investigations to replicate and extend these results are necessary, particularly because our analysis was limited by the study design, such that data on history of cigarette smoking and exposure to dietary heterocyclic amines and mutagens were only available for half our study population.

There is a well-established correlation between *NAT2* genotype and acetylation phenotype [13,15]. NAT2 rapid-acetylators demonstrate increased *O*-acetylation activity, thereby increasing the bioactivation of aromatic and heterocyclic amines and potential formation of DNA adducts. The genotype–phenotype relationship for NAT1 is less clear [13]. *NAT1*10* has been linked to increased *O*-acetylation activity in some studies [30], but not in others [31,32].

Three previous smaller epidemiologic investigations found no association between *NAT1* or *NAT2* genotypes and NHL risk [16,18,19]. A fourth epidemiologic investigation, which used caffeine as a metabolic probe to determine acetylation status, also found no significant relationship between acetylation status and overall malignant lymphoma, but did suggest an association between the NAT2 slow-acetylator phenotype and low-grade lymphoma [17]. The *NAT1* and *NAT2* genotype frequencies in our controls were similar to the control populations in previous studies. If genetic variations in *NAT1* and *NAT2* do modulate NHL risk, conflicting study results could arise due to variations among different study populations in the levels of exposure to *NAT1*/*NAT2* substrates [15]. Previous studies might have also had genotype misclassification because methods that allow sufficient distinction of the *NAT1*10* allele from other *NAT1* alleles have become available only recently (e.g. *NAT1*14A*); prior methods only detected the wild-type *NAT2*4* allele and a small number of variant *NAT2* alleles [15]. The accuracy of our genotyping data for individual SNPs and our method for assignment of *NAT1* and *NAT2* alleles therefore minimized such misclassification. Although our study population is the largest published to date, replication of our results is warranted in other large epidemiologic studies that distinguish between individuals with one versus two copies of the *NAT1*10* allele and between individuals with *NAT2* intermediate- versus rapid-acetylator phenotype, which was important in our data.

The distribution and levels of NAT expression in humans are tissue specific. NAT1 is present in most tissues

throughout the body, whereas NAT2 is expressed predominantly in the liver and gastrointestinal tracts [33]. Correspondingly, the effect of exposure to aromatic and heterocyclic amines on cancer risk appears to be organ specific [15]. For example, NAT2 slow-acetylators are at increased risk for bladder cancer [34], whereas rapid-acetylators are at increased risk for colorectal cancer [35]. Moderate linkage disequilibrium exists between the *NAT1* and *NAT2* loci, and thus the genotypes are not independent of one another; however, the SNPs genotyped in the present study were not in strong linkage disequilibrium [36]. In our study population, *NAT1*10* was over-represented among NAT2 rapid-acetylators, which is consistent with previous reports [15,37]. Inclusion of both *NAT1* and *NAT2* in the same model did not materially alter (> 10%) the NHL risk estimates for *NAT1*10/*10* or *NAT2* rapid-acetylators, suggesting that our overall findings for *NAT1* and *NAT2* are not confounded by one another.

Although the present study comprised a population-based design, several limitations should be taken into account in the interpretation of our findings. Loss of information from eligible subjects due to death or refusal to participate could have biased our results, although survival bias was minimized by the use of rapid case reporting systems in all four study centers. It is also unlikely that participation bias would completely explain our findings because our results were similar when we restricted our analysis to the study center with the highest participation rates (Iowa), and because it is unlikely that genotype frequencies vary by willingness to participate [21]. In addition, the observed frequencies of the *NAT1*10/*10* genotype (5.5% among controls) and of homozygous *NAT2* rapid-acetylators (6% among controls) were similar to that expected in a predominantly Caucasian population in the USA [38]. Finally, information bias resulting from exposure misclassification is likely to have been non-differential, thus biasing our risk estimates towards the null.

In summary, our data provide evidence of differential susceptibility to NHL in relation to genetic variation in acetylation capacity, thereby suggesting a contributory role for carcinogenic aromatic and/or heterocyclic amines in the multi-factorial etiology of NHL. If our results are replicated in additional studies, future large-scale epidemiologic investigations of carcinogenic aromatic and heterocyclic amines that take into account variation in acetylation capacity are warranted.

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